treatment were used. At selected time intervals, cover slips were removed from the chamber and placed in vials containing cocktail prior to liquid scintillation counting.

Initial investigations indicated that abietic acid reduced volatility by salt formation rather than due to a true release-matrix effect. The simple fatty acids: acetic acid, lauric acid, myristic acid, palmitic acid, stearic acid and oleic acid were therefore investigated. These were co-dissolved with pyrimethanil at 1:1 and 1:2 molar ratios of pyrimethanil: acid, the pyrimethanil being maintained at 0·5 g litre^{-1}.

In addition to the fatty acids which were investigated for volatility redution, a large range of other organic acids were used to make pyrimethanil salts. These were isolated, characterised and formulated as simple WP formulations for biological evaluation against a range of pathogens.

3 Results and discussion

Volatility. As alluded to in the preceding section, matrix-forming materials, when used at commercially acceptable rates (for one-pack products), had minimal long-term impact on the volatilisation of pyrimethanil. However, salt formation with the high-melting-point abietic acid indicated salt formation to be a viable approach. Some salts with fatty acids showed considerable potential to reduce loss due to volatility (Table 1). Predictably, the use of acetic acid had no impact on volatility, as the resultant salt did not differ greatly in molecular weight from pyrimethanil, but salts with the longer-chain acids showed massive reduction in volatilisation of pyrimethanil.

Biological activity. A range of organic salts were investigated, both by treating the salt as a new active ingredient and by adjusting doses to pyrimethanil-equivalence. Activity benefits were demonstrated in both cases.

When tested against *Erysiphe graminis* DC on wheat using a standard seven-day protectant protocol, the pyrimethanil 2*H*-1-benzopyran-3-carboxylate salt (50 g kg^{-1} WP formulation) showed nearly 80% disease control at 100 g per salt compared with less than 30% control by the corresponding pyrimethanil 50 g kg^{-1} WP. Similarly, in a 21-day protectant test against *Leptosphaeria nodorum* Muell., both this salt and a 50 g kg^{-1} WP of dipyrimethanil phthalate showed two-fold greater activity at the same dose.

TABLE 1

Surface Recovery of [^{14}C]-Pyrimethanil 2 h after Application to a Glass Surface in Combination with Fatty Acids at a 1:1 Molar Ratio

Fatty acid	*Recovery (% of radioactivity applied)*
None	3·1
Oleic	77·9
Lauric	66·9
Myristic	71·9
Palmitic	61·9

4 Conclusions

It has been shown that formation of organic salts of pyrimethanil greatly reduces its potential loss by volatilisation. In addition, increased activity against a selection of pathogens has been achieved which may be related to reduced loss. However, differing physicochemical properties of the salts could also influence their 'availability' to the required target site, and may also be a hindrance to formulation. For example, if the organic acid is too water-soluble, an SC formulation is not viable. In addition, the salt of such an acid (e.g. saccharin) can rapidly decompose upon addition of a dry formulation to the spray tank, resulting in such physical problems as flocculation and mixing incompatibility. However, in the case of lipophilic ion-pairs, advantages have been gained. For example, the oleate salt is a low-viscosity liquid which is readily emulsifiable, offering new formulation opportunities in EC, EW and SE formulations and co-formulations. Similar advantages have been noted for a new ester of fluroxypyr.[3]

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Inhibitors of Appressorium Formation in *Magnaporthe grisea*: a New Approach to Control Rice Blast Disease

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Abstract: Fungi were screened for the production of inhibitors of appressorium formation in germinating conidiospores of *Magnaporthe grisea* on inductive and non-inductive surfaces. Bioactivity-guided isolation yielded glisoprenins A, C, D and E from *Gliocladium roseum* and oleic acid from three fungi. The glisoprenins were active only on a hydrophobic surface, whereas oleic acid inhibited appressorium formation on a hydrophilic surface when 1,16-hexadecanediol, but not 8-(4-chlorophenylthio)adenosine-3′,5′-monophosphate, was used as inducer. The inhibition by glisoprenins could be reversed competitively by 1,2-dioctanoylglycerol but not by 1-oleoyl-2-acetyl-glycerol, both effective activators of protein kinase C in mammalian cells. Other mono-unsaturated fatty acids also inhibited appressorium formation. The corresponding methyl esters were inactive. The results agree with previous findings that at least two signal-transducing pathways are involved in appressorium formation. In addition, the differences observed between fungal signalling via PKC and the pathway used in mammalian cells could be used for the search for new and selective fungicides for rice blast disease.

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Key words: Appressorium formation; glisoprenins; fatty acids; *Magnaporthe grisea*; signal transduction

1 Introduction

Many plant pathogenic fungi like *Magnaporthe grisea* (Herbert) Barr and *Colletotrichum* species, invade the host by means of melanised appressoria.[1,2] These infection structures are crucial for building up the hydrostatic pressure necessary for direct penetration of the leaf epidermal cells.[3] Prevention of appressoria formation could be a highly selective target for new fungicides to fight rice blast, the major disease of rice.

On landing on the leaf surface, the conidium germinates, and signals perceived from the host surface induce appressorium formation. It is known that appressorium formation can be induced by the hydrophobicity of the surface and that on a hydrophilic surface, host wax components and cAMP can act as inducers.[4–6] Therefore, test systems for screening were set up and the formation of appressoria on different surfaces in the presence and absence of inducers and extracts obtained from submerged cultures of fungi was followed. From the 300 extracts obtained from submerged cultures of fungi tested, four exhibited inhibitory activity. Since this is the first time that such a screening has been carried out, the active compounds from all four fungi were isolated and characterised.

2 Experimental

Organisms used. Magnaporthe grisea P2 was obtained from Dr B. Speakman, BASF AG, Ludwigshafen. It was cultivated as previously described.[7]

Test systems for appressorium formation. Appressorium formation was followed as reported previously.[8] Fatty acids were added dissolved in methanol at a final solvent concentration of <20 ml litre^{-1}. No effects of solvent on germ tube elongation or appressorium formation were observed at this concentration. Experiments were performed in triplicate and 100 conidia were evaluated in each test. Compounds were tested at 0·5, 1, 2, 4, 6, 8, 10, 15, 20, 25, 40, 50, 80 and 100 mg litre^{-1}.

3 Results

The fungi producing inhibitors of appressorium formation were identified as *Gliocladium roseum* Bainier, strain HA 190-95, *Dasyscyphus* sp. A23-96, *Hypocrea* sp. A20-96 and *Hericium ramosum* TA76020. Bioactivity-guided fractionation yielded glisoprenins A, C, D and E from HA190-95, all four of which were active only on a hydrophobic surface.[7,8] Whereas glisoprenin A had been already known from a *Gliocladium* species,[9] compounds C, D and E were new.[7,10] The effects of glisoprenins could be reversed by dioctanoylglycerol but not by 1-oleyl-2-acetyl-glycerol,[11] both known activators of protein kinase C in mammalian cells,[12] indicating that there are differences between fungal and animal cells in the signal pathway via PKC. Whether these might be a selective target for fungicides could be worth investigation. In agreement with two separate signal pathways and the hydrophobic signalling proceeding via PKC is also the observation that dioctanoylglycerol induced appressorium formation on a hydrophilic surface, whereas 1-oleyl-2-acetyl-glycerol was again inactive. That PKC plays an important role in appressorium formation has also been reported by others.[13] The active principle in the mycelial extracts from the other fungi was oleic acid. Therefore, other fatty acids and their derivatives were tested. Saturated fatty acids with chain lengths of 16, 18 or 20 carbon atoms were not active. Mono-unsaturated acids, irrespective of the configuration of the double bond, were inhibitory. The corresponding methyl esters had no effect. Linoleic acid, with two double bonds, was not active. In Table 1 the concentrations of glisoprenins and fatty acids that reduced appressorium formation by $80(\pm 5)\%$ are given. Oleic acid and elaidic acid interfered with the induction by 1,16-hexadecanediol but not with cAMP. It has been reported that the induction by chemical signals proceeds via cAMP,[1,14] therefore the fatty acids should interfere upstream of protein kinase A. How the effects of fatty acids on a hydrophobic surface should be interpreted is at present unknown.

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TABLE 1
Inhibition of Appressorium Formation in *Magnaporthe grisea* by Glisoprenins A, C, D and E and Fatty Acids on a Hydrophobic (A) and Hydrophilic (B, C) Surface

Compound	AIC_{80}[a] (mg litre^{-1}) A[b]	B[c]	C[d]
Glisoprenin A or C or D	4	>100	>100
Glisoprenin E	40	>100	>100
Palmitoleic acid	10	10	>100
Petroselinic acid	10	20	>100
Petroselaidic acid	20	20	>100
Oleic acid	20	2	>100
Elaidic acid	20	1	>100
cis-Vaccenic acid	20	20	>100
trans-Vaccenic acid	>100	10	>100

[a] AIC_{80}: Concentration at which appressorium formation was inhibited by 80(±5)%.
[b] GelBond sheet, control: 95·6(±2·4)% of the germinating conidia formed appressoria.
[c] Induction with 0·2 mg litre^{-1} 1,16-hexadecanediol: 91·4(±3.6)% of the germinated conidia formed appressoria.
[d] Induction with 25 mg litre^{-1} chlorophenylthio-cAMP 94·6(±2·1)% of the germinated conidia formed appressoria.

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A New Type of Plant Activator: Thieno[2,3-*d*][1,2,3]thiadiazole-6-carboxylic Acid Derivatives

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Abstract: A short and efficient synthesis of a series of the title compounds is presented starting with methylenebutanedioic acid and thioacetic acid. Using the Hurd–Mori reaction in the key step, the optimised reaction sequence allows the large-scale preparation of this new type of plant activator in a few steps with a high overall yield. Additional functionalisation of the 5-position via directed *ortho*-lithiation methodology is also described.

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Key words: plant activators; systemic acquired resistance; chemically induced resistance; thieno[2,3-*d*][1,2,3]thiadiazoles; Hurd–Mori reaction; directed *ortho*-lithiation

It has long been known that plants can develop a long-lasting broad-spectrum resistance against subsequent infections when locally infected with pathogens. In the course of extensive studies of this phenomenon it was discovered that induction of disease resistance in plants, called 'systemic acquired resistance' (SAR), can also be triggered by selected organic compounds which today are known as plant activators, examples being 2,6-

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